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Biochimica et Biophysica Acta 1772 (2007) 263–271

Biochimica et Biophysica Acta BBA

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Review

Non-viral gene therapy for Duchenne muscular dystrophy: Progress and challenges

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Received 29 May 2006; received in revised form 24 July 2006; accepted 25 July 2006
Available online 28 July 2006

Abstract

Duchenne muscular dystrophy (DMD) is one of the most common lethal, hereditary diseases of childhood. Since the identification of the genetic basis of this disorder, there has been the hope that a cure would be developed in the form of gene therapy. This has yet to be realized, but many different gene therapy approaches have seen dramatic advances in recent years. Although viral-mediated gene therapy has been at the forefront of the field, several non-viral gene therapy approaches have been applied to animal and cellular models of DMD. These include plasmid-mediated gene delivery, antisense-mediated exon skipping, and oligonucleotide-mediated gene editing. In the past several years, non-viral gene therapy has moved from the laboratory to the clinic. Advances in vector design, formulation, and delivery are likely to lead to even more rapid advances in the coming decade. Given the relative simplicity, safety, and cost-effectiveness of these methodologies, non-viral gene therapy continues to have great promise for future gene therapy approaches to the treatment of DMD.
Published by Elsevier B.V.

Keywords: Muscular dystrophy; Dystrophin; Antisense oligonucleotide; Plasmid gene therapy; Gene editing

1. Introduction

Duchenne muscular dystrophy (DMD) is a devastating disorder of childhood, leading to loss of ambulation during the first decade of life, progressive weakness, and death usually in the twenties [1]. The molecular basis of this disease is an absence of the dystrophin protein [2]. At the genetic level, dystrophin deficiency arises from mutations in the dystrophin gene, but the range of mutations is enormous both in terms of types of mutations (deletions, duplications, inversions, point mutations) and in terms of the location of the mutation along the 2.5 Mb dystrophin gene [3,4]. Despite the advances in molecular diagnoses, accepted treatment modalities remain limited to corticosteroids and physiotherapy, and treatment is largely supportive [1,5].

Experimental approaches for the treatment of DMD have greatly expanded in the past decade. Gene and cell therapies continue to be at the forefront of those approaches, but pharmacologic and hybrid methods have received considerable attention [5]. Traditionally, gene therapy has been divided into viral and non-viral, although this distinction also can be blurred as technologies are combined. Within the category of non-viral gene therapy methodologies, three different approaches have been pursued with respect to DMD. These are: 1) the use of plasmid DNA to deliver dystrophin cDNA constructs; 2) the use of antisense oligonucleotides (AONs) to induce exon skipping of the dystrophin pre-mRNA to convert and out-of-frame transcript to an in-frame transcript; and 3) the use of RNA–DNA chimeric oligonucleotides (RDOs) or oligodeoxynucleotides (ODNs) for genome editing or gene correction to alter single bases in the dystrophin gene to correct point mutations or to alter mRNA splicing. Reviews of the each of these technologies have been published in manuscript and chapter form [6–10]. The focus of this review is to summarize the most recent

Abbreviations: AON, antisense oligonucleotide; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; ODN, oligodeoxynucleotides; RDO, RNA/DNA chimeric oligonucleotide

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0925-4439/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.bbadis.2006.07.009

technological developments in each of these fields, to highlight the most promising and important research directions, to enumerate specific hurdles and challenges faced by each technology, and to discuss the status of nascent clinical trials in humans.

Although plasmid-mediated gene delivery, AON-induced exon skipping, and oligonucleotide-mediated genome editing have unique promises and challenges (Table 1 and as described below), there are also commonalities in terms of advantages and disadvantages when compared to viral-mediated gene therapy approaches. In general, given current technologies, it is considered that non-viral vectors will represent a less expensive and safer mode of therapy. The cost of production of plasmids and oligonucleotides has dramatically fallen in the past decade, whereas the generation of clinical grade viral stocks has inherently high costs. Many safety issues remain to be determined for all forms of gene therapy, but concerns over potential adverse effects of viral-mediated gene therapy have been realized in terms of morbidity and mortality in human clinical trials [11,12]. Despite the theoretical advantages of non-viral vectors in terms of safety, caution will be warranted as these approaches also move from laboratory animals to humans.

Perhaps the most obvious and important disadvantage of non-viral vectors is one of delivery, and here delivery is meant to include everything from the distribution via the circulation of the therapeutic vector to all skeletal muscles and the heart, the process by which the vectors leave the blood to reach muscle cells, the mechanisms by which the vectors enter the cells, and finally the processes by which the vectors reach the intracellular target. Although non-viral vector development can improve upon the specificity and efficiency of these phases of delivery, viruses accomplish all of these aspects of delivery without any help from the investigator. As such, important recent developments that could apply to many or all non-viral approaches for DMD include advances in intra-arterial and intravenous delivery protocols [13–17], adjunct treatments to promote vascular permeability to facilitate the transfer of vectors (including viral vectors) from the circulation to the tissue bed [18–20], and formulations to enhance stability and cellular uptake of non-viral vectors [21–23]. Although some of the most promising results have been obtained by intra-arterial injections, and even though multiple arterial injections to reach a larger subset of muscles of a patient would be a viable approach, having an intravenous deliver system would be far less invasive.

Table 1

	Plasmid-mediated gene delivery	Antisense-induced exon skipping	Oligonucleotide-mediated gene editing
Efficiency of inducing dystrophin expression [a]	+++	+++	+
Duration of effect [b]	+++	+	++++
Uptake of vector after delivery to tissue [c]	++	++++	++++
Applicability regardless of mutation [d]	++++	++	+

[a] Efficiency is a difficult parameter to compare because measures of efficiency vary widely. Based on in vivo type experiments in which the primary outcome is the generation of dystrophin-positive fibers, both plasmid-mediated gene delivery and AON-induced exon skipping yield much higher numbers than oligonucleotide-mediated gene editing. Of course, none of these measures are normalized to vector number or concentration in the cell.

[b] AON-induced exon skipping results in transient dystrophin expression, plasmid-mediated gene delivery results on sustained dystrophin expression, and oligonucleotide-mediated gene editing results in permanent correction.

[c] In general, the smaller oligonucleotides used for exon skipping and gene editing are more readily taken up into muscle fibers that are large plasmids, even when plasmids are formulated with agents to enhance uptake.

[d] The expression of dystrophin from a plasmid is an effective treatment regardless of the nature of the endogenous gene mutation. By contrast, both AON-induced exon skipping and oligonucleotide-mediated gene editing require vectors specifically designed for the underlying mutation. However, certain AONs will be applicable to a number of different deletion mutations.

Clearly immunological challenges are faced by all gene therapy approaches. The possibility of an immune response directed against the therapeutic protein is always a concern when the individual is deficient for that protein. In the case of dystrophin, both humoral and cellular immune responses have been detected in dystrophin-deficient mdx mice following delivery of full-length dystrophin cDNAs, although not following the delivery of a minidystrophin construct [24,25]. This suggests that the response in an individual patient might depend on the specific epitopes in the protein expressed, either from an exogenous cDNA or from modulation of the endogenous gene or transcript. Studies of plasmid-mediated gene expression indicate that the likelihood of developing immune responses against the novel protein may be ameliorated by using muscle-specific promoters, and that even transient immunosuppression around the time of plasmid delivery can limit the development of an immune response [26].

The other major immunological issue is of course the possibility of an immune response against the vector itself. Although certainly safer than viral vectors in this regard, non-viral gene therapy vectors still have the potential to induce immune responses, particularly if they contain bacterial DNA sequences with unmethylated CpG motifs [27]. Although intramuscular injections of plasmid or double-stranded oligonucleotides bearing such sequences may result in local inflammatory responses [28], and repeated injections of plasmid have been shown to result in increases in circulating levels of anti-DNA antibodies [29], there have been no reports of autoimmune diseases resulting from such treatments. Whether low levels of circulating anti-DNA antibodies generated by treatment with non-viral vectors could attenuate subsequent systemic treatments with those vectors remains to be determined.

Finally, there is one other unknown that haunts the field of gene therapy for DMD, and that is the uncertainty of the normal turnover rate for myonuclei. Estimates of myonuclear turnover rate have been determined in certain muscle groups in experimental animals [30,31], but there are no reliable data on the turnover of myonuclei in the large muscles of the trunk and limbs in humans. Most technologies focus on the delivery of the gene

therapy vector to the differentiated myofiber, and the efficiency of targeting to the endogenous muscle stem cell, the satellite cell, is rarely examined. In the worst case scenario, myonuclear turnover rate is high and the gene therapy approach fails to target satellite cells. In that case, the therapeutic effect will necessarily be transient, as "corrected" myonuclei are replaced by nuclei from satellite cells that do not carry the therapeutic vector. However, examples of both viral and non-viral vectors capable of transducing satellite cells after intramuscular injections have been reported [32,33]. Different pseudotyped lentiviral vectors differentially transduce satellite cells and muscle fibers [33], thus demonstrating that satellite cells can be specifically targeted. For non-viral technologies, such targeting would necessarily involve a carrier that is modified so as to bind specifically to muscle cells, analogous to methods that have been proposed for modifying viral vectors to target muscle [34]. Whether such kinds of modifications could specifically target satellite cells remains to be determined. The importance of satellite cell targeting by gene therapy vectors for sustained therapeutic efficacy may ultimately await empirical determination in clinical trials or even long-term clinical applications since studies in animals may poorly reflect the dynamics of myonuclear turnover in humans.

Solutions to these common challenges will lead to advances in all aspects of non-viral gene therapy approaches to DMD. Still, each methodology will also have its "private" hurdles as well. In the following sections, the different non-viral gene therapy approaches that are currently under active investigation for DMD are reviewed, with attention to current challenges and future directions.

2. Non-viral gene therapy technologies and experimental therapeutics for DMD

2.1. Plasmid-mediated gene delivery

Plasmid-mediated gene therapy is based on the observation made over a decade ago that naked plasmid DNA, delivered to skeletal muscle by direct injection, is taken up in the myofiber where plasmid genes are expressed [35]. Various parameters such as plasmid size, concentration, and promoter sequences have been tested to optimize gene delivery options [36–39]. Advantages of plasmid gene therapy include already existing scaled-up production processes, applicability to DMD patients regardless of mutation, and simplicity. Following the initial observations that dystrophin protein could be expressed in muscles of mdx mice after simple intramuscular injections of plasmid [40,41], much effort was devoted to improved efficacy of distribution, uptake, and expression of injected plasmids. Improvements have involved adjunctive treatments, notably using electroporation and ultrasound, co-injecting enzymes such as hyaluronidase, and using non-ionic carriers for plasmid DNA [42–48]. Of these, electroporation leads to the most dramatic enhancement of gene expression, although there is significant muscle damage that results [49].

Multiple intramuscular injections with electroporation would, however, be an extremely laborious and invasive method for the treatment of all muscles of a patient, not to mention the challenge of delivering plasmid to the heart and diaphragm by this method. As such, attention has focused more recently on regional or systemic delivery of plasmids. Intra-arterial delivery of plasmid in mdx mice resulted in expression of dystrophin in 1–5% of fibers in the injected limb [13]. This approach has been used to deliver plasmids to limb muscles in non-human primates with even higher efficiencies [26].

Given the encouraging results of plasmid-mediated gene therapy in animals combined with technological advances and the potential of systemic delivery, this experimental therapeutic approach has moved from the laboratory to the clinic. A phase I clinical trial was initiated recently with the overall design of either a single injection or two injections of plasmid containing full-length dystrophin cDNA into single muscles of a group of 9 boys with DMD or Becker muscular dystrophy (BMD) [50]. The trial was designed to test for any adverse effects of low dose intramuscular plasmid injections and for immunological responses. No adverse effects were noted. In follow up biopsies 3 weeks after injections, dystrophin protein was detected in only 6 of the 9 boys and in only a small percentage of fibers, but no signs of an immunological response was observed [51,52].

As clinical trials progress, studies designed to improve the efficiency and efficacy of plasmid-mediated gene therapy continue. An unresolved issue for treatment of humans with DMD is the expected duration of plasmid gene expression. In mice, transgene expression can be detected for many months after direct intramuscular injection in normal muscle. However, there is clearly a continual loss of plasmid over this time [53]. In humans, sustained expression would be necessary for years and decades. Therefore, any technological advance that would lead to plasmid persistence is likely to be an important adjunct to plasmid-mediated gene therapy for DMD. Recently, a targeted plasmid integration approach has been used to address this issue (Fig. 1). This approach is based on the finding that the co-expression of an integrase with a plasmid that contains one of the integrase recognition sequences allows for integration of the plasmid into the mammalian genome [54]. This integrase technology was applied to the delivery of dystrophin-expressing plasmids to mdx muscle, and plasmid integration resulted in more sustained dystrophin expression [55]. An increased expression of dystrophin was seen at each time point examined, with the difference increasing with time, demonstrating the importance of integration for sustained plasmid gene expression [55]. The integrase-mediated enhancement of long-term gene expression in skeletal muscle was also evident when tested with plasmids expressing vascular endothelial growth factor [56].

Ideally, this technology would be truly site-specific, introducing the dystrophin plasmid into a single site in the genome, but the currently used integrases clearly integrate at multiple sites even though there are clear 'hot spots' [55,57]. Therefore, there is the theoretical risk of insertional mutagenesis, which may have resulted in the development of leukemias in three patients in a clinical trial of retroviral-mediated gene therapy for X-linked severe combined immune deficiency [11]. However, not only does recent evidence suggest that those leukemias may have resulted from expression of the therapeutic

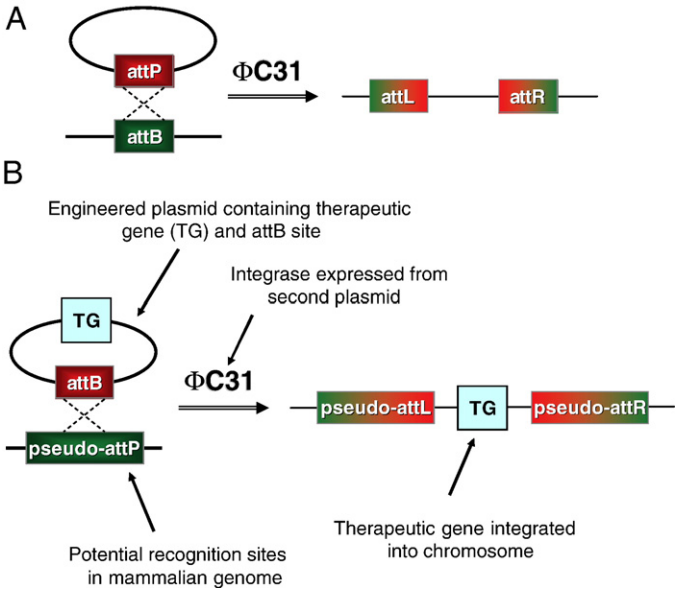

Fig. 1. Targeted plasmid integration to enhance plasmid-mediated gene delivery. (A) Native function of ϕC31 integrase. This integrase is a phage product that catalyzes the integration of the phage genome, containing a unique attP sequence, into the bacterial genome, containing a unique attB sequence. Because these two sequences are different, the resulting sites following recombination are distinct (one attL sequence and one attR sequence) and different from the attB and attP sequences, rendering this reaction unidirectional. (B) Co-opting ϕC31 integrase for gene therapy. By designing plasmids with attB sequences and delivering a second plasmid encoding the ϕC31 integrase, sites within the mammalian genome with sufficient sequence homology to attP sites allow for the integrase to catalyze the recombination and integration of the therapeutic plasmid into the mammalian genome.

gene as opposed to gene disruption from vector integration [58], but this risk is of course very low when postmitotic myonuclei are the targets as opposed to hematopoietic progenitors.

Finally, an issue that has come to light in studies of plasmid-mediated gene therapy, even though this would apply to all gene therapy approaches, is the distribution of the vector and thus the therapeutic protein along the length of muscle fibers in a targeted muscle. Many studies of long-term efficacy of plasmid-mediated gene delivery have been quantitated by measuring the number of muscle fibers that express the transgene over time [41,59]. This unfortunately underestimates protein loss; direct comparisons reveal that the total protein levels can be declining while the number of fibers that stain positively for the protein remains relatively constant since fibers with lower, but still detectable, levels of transgene expression are scored as positive [53]. The situation is even more critical in dystrophic muscle because of the issue of segmental necrosis. Any fiber segment that does not express therapeutic levels of dystrophin is susceptible to degeneration which can then encroach upon segments with marginally therapeutic levels. Thus, any fiber in which dystrophin is not expressed at therapeutic levels along its length will eventually succumb. This was clear in studies of integrase-enhancement of plasmid gene therapy where it was found that the increased dystrophin expression and persistence by integration results in a higher number of fibers with therapeutic dystrophin levels along their entire lengths [55]. This finding highlights the importance of quantifying dystrophin expression not only in the muscle cross-sections but also along the longitudinal axis of the muscle.

2.2. AON-induced exon skipping

AONs are composed of RNA residues or homologs and have been primarily used to block translation by binding to mRNA and leading to its degradation [60]. However, it was also noted that AONs targeted to splice sites or splicing regulatory regions of pre-mRNA could alter the splicing of that transcript, presumably by interfering with the normal splicing machinery [61]. The rationale for the therapeutic benefit of modifications of dystrophin transcript splicing to treat DMD is based on the fact that most cases of DMD are caused by deletions in the dystrophin gene that lead to frame-shift mutations in the transcript [62]. Deletions that do not cause a frame shift but simply an internally deleted protein tend to cause the milder, allelic disease, BMD. Therefore, it was hypothesized that if one could alter the splicing of a DMD gene to produce a shorter but in-frame transcript that this would be a method to convert a severe DMD phenotype into a milder BMD phenotype (Fig. 2).

Proof-of-principle studies were initially published about a decade ago [63,64]. Progress was rapid in terms of improved vector design, target choice, and oligonucleotide chemistries, all leading to more efficient and effective exon skipping [65–68]. Dystrophin expression was demonstrated using AONs delivered to dystrophin-deficient muscle cells in vivo in the mdx mouse or in vitro [69–71]. Further developments toward clinical application have included methodologies, as mentioned above, to enhance the uptake of AONs in mouse muscle in vivo and the use of systemic delivery methods to begin to develop a clinically feasible delivery approach [17,21–23].

The application of AONs to mediate exon skipping in DMD has progressed remarkably quickly from the laboratory to the clinic. Phase I clinical trials with AONs in humans are in early stages in Britain and Holland [72]. These will involve intramuscular injections of AONs to test for safety, and it is likely that some measures of efficacy will be included in the analysis. If these phase I trials demonstrate safety, the challenge will be to scale those up for targeting large muscles, whole limbs, relatively inaccessible targets like the diaphragm and heart, and ultimately the entire body musculature. It will be at that point that the translation of systemic delivery methodologies from laboratory animals to humans will be the major challenge.

At the same time, the development of new generations of AONs could further enhance therapeutic efficacy. Currently, the chemistries that are being tested are 2′O-methyl phosphorothioate and morpholino-phosphorodiamidate AONs (Fig. 3). Other chemistries that have been tested include AONs containing peptide nucleic acids, locked nucleic acid, and ethylene-bridged nucleic acid backbones [67,73]. Direct comparisons in terms of efficacy and toxicity in vivo will be required to determine the optimal chemistry for clinical trials.

One of the challenges of AON therapy is that it is not a ‘one-size-fits-all’ approach in the same way that gene delivery is. In order for an AON approach to be applicable, there needs to be an

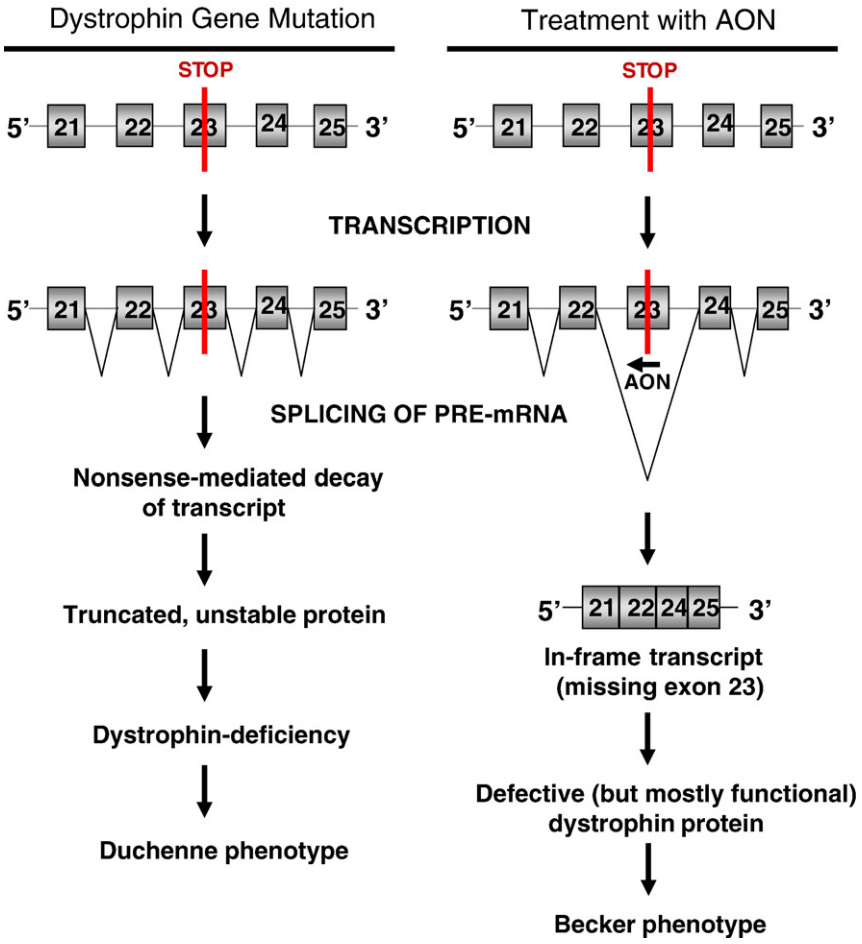

Fig. 2. Molecular mechanism of AON-induced exon skipping to convert a Duchenne phenotype into a Becker phenotype. The nonsense mutation of the mdx mouse is shown as an example, but the same principles apply for deletions that generate out-of-frame transcripts in which skipping one or more exons can generate an in-frame transcript. An AON targeted to exonic splice enhancer sequences is shown. AONs targeted to 5′ or 3′ splice sites are also effective in inducing skipping of the specific exon.

accurate molecular diagnosis, a rational plan for skipping one or more exons to produce an in-frame transcript, evidence that targeting AONs to specific sequences will result in the desired transcript, and finally the design of AON vectors specifically for that particular mutation. This seemingly major hurdle of customized therapy is partially obviated by the fact there are certain deletions that are very common and that a relatively small number of deletions represent a majority of cases of DMD. Thus, a limited number of AONs will theoretically be effective in a majority of DMD patients [74]. In those cases where skipping of two exons would be necessary to generate an in-frame transcript, multiple AON delivery could be used, as has been demonstrated [75]. Of course, without a defined molecular diagnosis, AON therapy is not applicable.

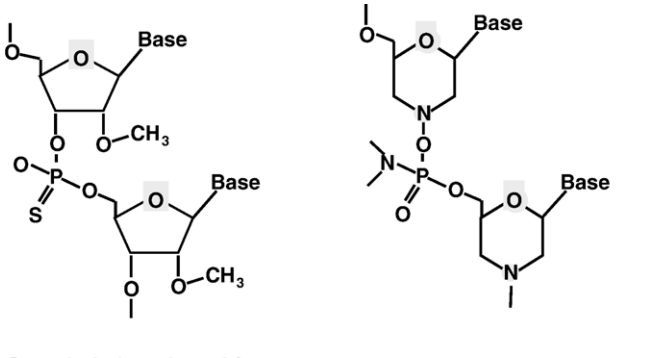

Fig. 3. Chemical structures of modified bases used in AON design. The two chemical structures shown are the most widely studied and are the modifications that have been proposed to be tested in human clinical trials.

Finally, one of the intrinsic limitations of AON-mediated exon skipping is the fact that the therapeutic effect is transient. AONs are delivered as oligonucleotides which are unstable in the cell. In mice, detectable levels of dystrophin expression were obtained by sequential AON injections, but levels declined with a half-life of approximately 2–4 months [22], and this could even be an overestimate of AON stability as it may be more of a reflection of dystrophin protein stability. Even so, protein persistence is the key determinant of the duration of therapeutic efficacy and is thus the meaningful measure. As currently configured, this would mean that AON therapy would require multiple treatments each year. While not untenable, this is clearly a serious hurdle to overcome. In mice, investigators have combined AON technology with viral expression

approaches to achieve sustained expression of antisense vectors and long-term exon skipping [76–78]. However, this introduces the challenges of viral gene therapy, and there is no clear advantage of delivering an antisense vector as opposed to delivering a dystrophin construct itself. Another possibility on the horizon is to introduce a short cDNA encoding U7-small nuclear RNA to achieve sustained exon skipping [79]. Thus, the technological advances that will be critical for long-term AON therapy will come in the form of oligonucleotide modification or formulation that promotes very long-term stability or sustained in vivo delivery.

2.3. Oligonucleotide-mediated genome editing

The use of targeting oligonucleotides to induce single base changes in genomic DNA developed from initial studies in homologous pairing and repair activities in bacteria and simple eukaryotes [80,81]. The theoretical basis for this approach is that engineered mismatches between the targeting vector and the genome sequence could, through endogenous DNA repair mechanisms, potentially promote targeted single base pair changes in genomic DNA [82–85]. The possibility that such genome editing potential could be applied to the correction of point mutations that cause disease was compelling (Fig. 4). As such, this technology has been applied to a wide variety of models of diseases arising from point mutations in different genes [86–88].

Upwards of 15% of patients with DMD have point mutations as the cause of dystrophin deficiency [4]. Fortuitously for this particular application, the most commonly used animal model of DMD, the mdx mouse, also has a point mutation that results in a stop codon as the cause of dystrophin deficiency. Therefore, the mdx mouse has served as an excellent model with which to test oligonucleotide-mediated dystrophin gene editing. Initial studies using RDOs and subsequent studies using ODNs demonstrated that the point mutation in the mdx dystrophin gene can indeed be corrected by targeting oligonucleotides [32,89,90]. Correction was demonstrated at the genomic and transcript levels, and restoration of dystrophin protein expression was observed both in vivo and in vitro. In addition, RDOs were shown to be able to correct the point mutation in a canine model of DMD [91].

The application of this type of technology to diseases that result from point mutations is clear, but broader applications have been envisioned whereby single bases in splice sites could be altered at the genomic level, thereby inducing alternate splicing to effect the same result as that achieved by AONs, namely the generation of an in-frame transcript from an out-of-frame transcript. This approach has been demonstrated in the mdx mouse by inducing skipping of the exon that contains the point mutation [92]. As described for AONs, oligonucleotide-mediated gene editing also requires individualization since the targeted base will depend on the location of the point mutation or the particular splice site to be targeted.

Other than the challenges common to all non-viral methodologies discussed in Introduction, the major challenge for oligonucleotide-mediated gene editing is the low efficiency of

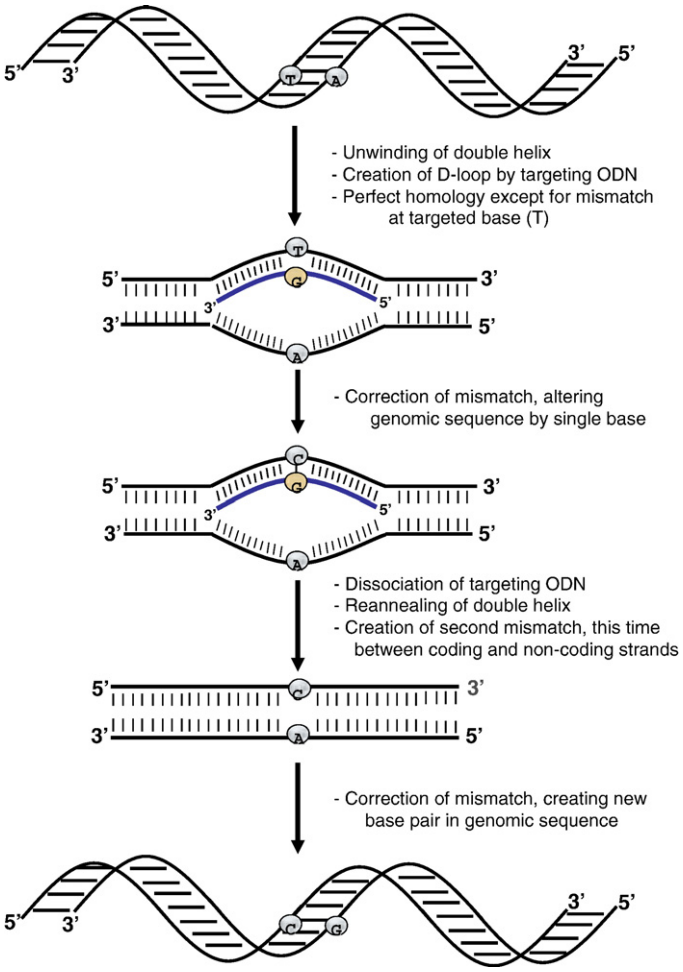

Fig. 4. Molecular mechanism of ODN-mediated gene editing. A hypothetical T–A base pair mutation, for which the wild-type pair is a G–C, is illustrated. The targeting ODN, perfectly complementary to a region of one strand of the gene of interest except for a single mismatch with the mutant base, is first involved in a pairing reaction in which the ODN inserts into the double helical structure. The mismatch between the ODN and the genomic sequence induces endogenous repair mechanisms to correct the mismatch, and the therapeutic effect occurs when the genomic base is altered. Following dissociation of the ODN, the reannealing of the double strand leads to another mismatch. Again, the therapeutic effect occurs when the mutant base is now corrected, leading to a permanent conversion of the mutant T–A pair to the wild-type G–C pair.

this technology with current vectors that has been observed a variety of different disease applications [86,87]. The was the case with the first generation RDO vectors, but has also been found with the second generation vectors, the ODNs, which yield more consistent results [85,93]. Both RDOs and ODNs have been shown to be effective in vitro and in vivo to induce single base changes, but the efficiencies have generally been in the 1–5% range, with higher efficiencies being observed only under specific conditions [86,89,90,92,94–99]. Therefore, current research is focusing on the enhancement of the molecular mechanisms that mediate the gene editing, and these studies include both modifications of the oligonucleotides as well as enzyme systems and cellular processes involved in the repair mechanism [100–102]. Improvements in genome editing efficiency is currently the most important hurdle for the

consideration of a phase I clinical trial of ODNs in humans similar to those conducted or planned for plasmids and AONs.

Perhaps the greatest advantage of the gene editing approach is that it results in a permanent correction of the genetic defect in myonuclei. Therefore, the therapeutic benefit is sustained as long as the targeted myonuclei persist. The only decrement of therapeutic efficacy is that which would accompany the turnover of myonuclei and replacement by nuclei derived from satellite cells, as noted in Introduction. However, it is possible that even this could be less of an issue for oligonucleotide-mediated gene editing than for non-viral methodologies. Although not quantitative, it was shown that targeting oligonucleotides injected into skeletal muscles of mdx mice were capable of inducing gene correction in satellite cells subsequently isolated from those muscles [32]. To the extent that ODNs could be directed both to satellite cells as well as to myofibers, long-term permanent gene correction could be achieved.

3. Conclusions

Although the field of gene therapy has failed to live up to initial promise, the advances in the control of gene expression, delivery technologies for different vectors, and methods to avoid, reduce, or prevent immunologic responses to vectors and therapeutic proteins have benefited all aspects of gene therapy for all disease for which gene therapy is a viable option. Clearly, the simplicity of concept belies the complexity of application. It is likely that, even with the real possibility of finding a cure for DMD, advances are likely to be incremental. In the field of non-viral gene therapy for DMD, what is perhaps most remarkable is the rapid development of technologies that were little more than curiosities a decade ago. This is an important lesson for nascent technologies, including other forms of non-viral gene therapy that have yet to be applied to models of DMD, that are far behind other methodologies in terms of technical development. Unless relatively obscure technologies had been championed by dedicated investigators, the current state-of-the-art would be much more limited in scope. It is impossible to predict which curiosity of today will be in clinical trials a decade hence, and the lessons learned from the technological development in one area are likely to be of benefit to the broader field of gene therapy.

Acknowledgements

Supported by grants from the NIH (NIH Director's Pioneer Award) and the Muscular Dystrophy Association.

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